RESEARCH ARTICLE

Alteration of Leptin and Adiponectin in Multistep Colorectal Tumorigenesis

Jirakrit Saetang[1], Teeranut Boonpipattanapong[2], Anuwat Palanusont[2], Wanwisa Maneechay[3], Surasak Sangkhathat[1,2]*

Abstract

Background: There is an established link between obesity related metabolic derangement and colorectal cancer development. Recently, we developed a metabolic-colorectal cancer risk score. In this follow-up study, we studied its association with colorectal neoplasm by measuring two major metabolic syndrome biomarkers, leptin and adiponectin. Objectives: To evaluate the serum levels of leptin and adiponectin in patients with colorectal polyps and colorectal cancer and to determine any correlation with metabolic risk score. Results: In total, 130 individuals were studied: 30 controls without colonic pathology, 18 with colonic adenoma (CAP), and 82 with colorectal adenocarcinoma (CRC, 17 cases of T1-2 and 65 cases of T3-4). The metabolic risk scores in CAP and T1-2 CRC were higher than those in the controls and T3-4 CRC cases. There were no statistically significant differences in leptin levels among CAPs, CRCs, and controls. Both leptin and adiponectin levels reflected differences in body mass index and metabolic risk scores. Cases in the CAP group and early T-stage CRC groups had lower adiponectin levels (14.03 and 13.01 mg/ml, respectively) than the no polyps group (19.5mg/ml, p = 0.03). The average serum adiponectin level in the invasive cancer group (18.5 ng/ml) was comparable with that of the control group. Conclusions: The level of serum adiponectin was positively correlated with the metabolic risk score. Decreased serum adiponectin was significantly associated with the development of colorectal adenoma and early stage colorectal carcinoma.

Keywords: Colorectal cancer - metabolic risks - leptin - adiponectin

Asian Pac J Cancer Prev, **17 (4)**, 2119-2123

Introduction

Metabolic syndrome is a lifestyle-related health problem, and its incidence continues to grow in modern society (Doyle et al., 2012). The core feature of metabolic syndrome is visceral obesity, and its accompanying metabolic derangement includes insulin resistance, dyslipidemia, and hypertension (Alberti et al., 2006). Moreover, hyperuricemia and transaminitis are also detected in these patients (Wei et al., 2011, Singh et al., 2013, Bonakdaran and Kharaqani. 2014, Orannapalai et al., 2014). Apart from its known and unsurprising link to cardiovascular diseases, metabolic syndrome has also been shown to increase the risk of various cancers, including pancreatic, esophageal, and colorectal cancers (Doyle et al., 2012). Recently, we developed a metabolic risk score that predicts the chance of identifying colorectal polyps using metabolic parameters (Orannapalai et al., 2014).

Colorectal cancer is an epithelial cancer of the distal part of the gastrointestinal tract that is developed through the adenoma-carcinoma sequence (Arnold et al., 2005). In this process, the cancerous epithelium develops on top of a premalignant lesion, i.e., adenomatous polyp, which is an intermediate pathology that can be readily detected by colonoscopy. Visceral obesity and chronic inflammation are thought to contribute to the initiation of accelerated growth and tumor development within the colonic mucosa (Riondino et al., 2014). Cytokines released from adipose tissue, known as adipokines, are responsible for local tissue inflammation and growth promotion (Doyle et al., 2012, Vazzana et al., 2012). The two best known adipokines involved in cancer development are leptin and adiponectin (Zhang et al., 1994, Green et al., 1995).

Leptin, a 16 kDa peptide hormone primarily secreted by adipocytes, is encoded by the *LEP* gene on chromosome 7q31.3 (Zhang et al., 1994, Green et al., 1995, Prolo et al., 1998). The hormone's main function is to regulate energy homeostasis by means of anorexigenic neuron activation at the level of the hypothalamus (Cowley et al., 2001, Seoane et al., 2015). In the peripheral organs, leptin acts together with cholecystokinin to suppress food intake by the intestine (Attele et al., 2002) and promotes

*[1]Department of Biomedical Science, Faculty of Medicine, [2]Tumor Biology Research Unit, Faculty of Medicine, [3]Central Research Laboratory, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand *For correspondence: surasak.sa@psu.ac.th*

growth of colonic epithelial cells (Hardwick et al., 2001). Serum leptin levels are significantly correlated with body mass index (BMI) and fat composition (Lubkowska et al., 2015). Regarding colorectal cancer development, recent meta-analyses have concluded that there is a positive association between increased serum leptin and the risk of colorectal adenoma (Gialamas et al., 2013, Aleksandrova et al., 2014). A study in a cell culture model demonstrated that leptin stimulated proliferation and inhibited apoptosis of colorectal cancer cells through the phosphatidylinositol 3 kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway (Wang et al., 2012).

Adiponectin is a 28 kDa protein encoded by the adiponectin gene (3q27) and secreted exclusively by adipocytes (Kadowaki and Yamauchi, 2005). In contrast to leptin, serum adiponectin levels decrease with obesity and growing fat composition (Kadowaki and Yamauchi. 2005). Adiponectin provides an insulin-sensitizing function through its membrane receptor, AdipoR (Trujillo and Scherer. 2005, Yamauchi and Kadowaki, 2008). The hormone increases transmembrane glucose transport and muscle fat oxidation. Adiponectin also has anti-atherosclerotic functions through inhibition of adhesion molecules and inhibition of tumor necrosis factor (TNF)-alpha induced inflammation (Kadowaki and Yamauchi. 2005).

In cancers, adiponectin has been reported to induce apoptosis and inhibit proliferation of cancer cells through an AdipoRs/AMP-activated protein kinase (AMPK)/ mitogen activated protein kinase (MAPK) pathway (Dalamaga et al., 2012; Zhang et al., 2015). Recent studies have reported a reduction in serum adiponectin in colorectal adenoma and adenocarcinoma (Erarslan et al., 2009; Kumor et al., 2009; Gonullu et al., 2010; Nakajima et al., 2010), although these finding are controversial (Lukanova et al., 2006; Zekri et al., 2015).

The objective of this study was to evaluate the levels of leptin and adiponectin in the serum of patients with cancer associated colorectal polyps and various stages of colorectal carcinoma compared to controls without colorectal lesions. In addition, the correlation between the level of both adipokines and the metabolic risk scores was analyzed.

Materials and Methods

The research was approved by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University (EC 56-73-10-1-3).

Patients and blood collection

Frozen serum samples (-80°C) from patients who underwent a colonoscopy at the NKC Institute of Gastroenterology and Hepatology, Songklanagarind Hospital during the year 2012 and who were enrolled in our recent metabolic risk score study (Orannapalai et al., 2014) were retrieved under consent. The samples included 30 samples from individuals without colonic polyps and 20 samples from those with cancer-associated polyps (CAP), which were defined as colonic polyps that indicated a higher risk of colorectal adenocarcinoma, including elements of tubular, villous, tubulovillous, or serrated adenoma. Additional serum samples from newly diagnosed colorectal adenocarcinoma (CRC) cases were collected under informed consent at the date of pre-operative colonoscopy. The metabolic risk score in each individual was determined by a scoring system, as we previously described (Orannapalai et al., 2014). Data regarding the histopathological diagnosis and staging of CRC patients were retrieved from the hospital information system. Staging work-up in our CRC cases followed the recommendations of the American Joint Committee on Cancer (AJCC) (Greene, 2002). The lymph node ratio was defined as the number of positive lymph nodes divided by the number of all lymph nodes retrieved from the pathological specimen (Kritsanasakul et al., 2012). Cases with incomplete diagnostic data were excluded.

Quantitation of serum adipokines

Quantitation of serum leptin and adiponectin was performed at the Central Research Laboratory, Faculty of Medicine, Prince of Songkla University. Leptin and adiponectin levels were measured with the enzyme-linked immunosorbent assay (ELISA), using a Novex Leptin ELISA kit, Human KAC2281 (ThermoFisher Scientific, Inc., Boston, MA, USA) and a Novex Adiponectin ELISA kit, Human KHP0041 (ThermoFisher Scientific). All laboratory assays were performed according to the manufacturer's protocol.

Statistical analysis

Continuous data are presented as the means and ranges, and categorical data are presented as percentages, unless stated otherwise. Comparisons between groups were performed using the student t-test or Mann-Whitney U test, depending on the normality of their distribution. Comparisons of categorical data used the Chi-square test. The leptin to adiponectin ratio was defined as 1,000 X (amount of leptin per amount of adiponectin). To compare the mean value of each adipokine (leptin, adiponectin, and leptin/adiponectin ratio) with its mean value in the control group, Z-scores of the means in the three other groups were constructed using a formula (Z-score = [mean value of the group - mean score in the controls]/standard deviation). Except for construction of the graph in Figure 2, which used Microsoft Excel 2011, all statistical calculations used Stata 14.0 (StataCorp LP, College Station, TX, USA).

Results

Demographic data

The 130 subjects (64 females and 66 males) included 30 patients without colonic polyps, 18 patients with pathologically proven CAP, and 82 patients with CRC. The average age of the patients was 59.1 years (range 19-88 years). The average ages in the CAP (60.8 years) and CRC (62.4 years) groups were significantly higher than that in the 'no polyps' group ($p<0.01$). The average weight was 60.0 kg (36-96.8 kg), and the average BMI was 23.0 kg/m2 (14.4-35.9 kg/m^2). The average metabolic risk score was 1.72 overall; 1.77, 2.33, and 1.57 in the 'no polyps', CAP, and CRC groups, respectively. The distribution of

metabolic risk scores in each group is shown in Figure 1. Note that within the CRC group, the average score of the T1-T2 cases (2.29) was significantly higher than that of the T3-T4 cases (1.38, Mann-Whitney U test, $p < 0.01$).

Leptin and adiponectin levels in cancer associated polyps and colorectal cancer

The average leptin level of all 130 cases was 6.90 ng/ml (0.10-39.09 ng/ml). The leptin level increased as BMI and metabolic risk score increased. There was no significant difference in leptin levels between the CAP patients and the no polyp control group (Table 1). Male subjects had significantly lower average leptin levels than females.

The overall average adiponectin level was 17.43 mg/ml (1.28-44.30 mg/ml). Adiponectin level was negatively correlated with both BMI and metabolic risk score (Table 1). Male subjects had a significantly lower average adiponectin level than females. Cases in the CAP and early T stage CRC groups had lower average adiponectin levels (14.03 and 13.01 μg/ml) than the no polyps group (19.49 mg/ml, $p = 0.03$). In addition, the level of adiponectin was higher in CRC patients with rectal cancer than in colon cancer patients (Table 2).

When the leptin/adiponectin ratios were examined, the ratio was significantly different between those with a BMI lower than 23.4 kg/m^2 and those with a BMI greater than 23.4 kg/m^2. The ratio increased as metabolic risk increased, but this measure only was statistically significant when comparing the high metabolic risk group and the low risk group (Table 1).

Leptin and adiponectin levels change thorough the progression of colorectal cancer

When the CRC group was split into early (T1-T2) and progressing cancer (T3-T4) groups, we found that the adiponectin levels were significantly lower in the progressing cancer group than the early cancer group (Table 2). Changes in the opposite direction were observed in the leptin/adiponectin ratio. Z-scores for comparing the deviation of each of the three parameters from their base values (mean of controls) found that adiponectin changed

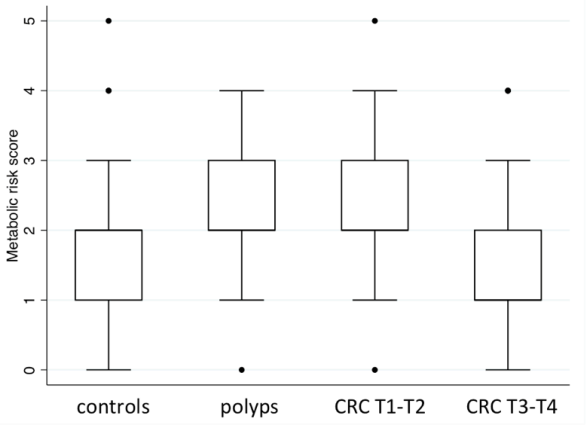

Figure 1. Box Plots of Metabolic Risk Scores in the Four Study Groups of Patients in this Study. CRC: colorectal cancer

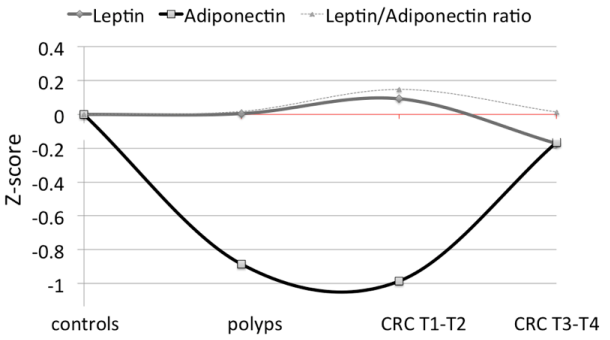

Figure 2. Z-scores of Leptin, Adiponectin, and the Leptin/Adiponectin Ratios in Polyps and CRC Groups Compared to Mean Values in the Control Group

Table 1. Leptin, Adiponectin Level and Leptin Adiponectin Ratio in 130 Cases Studied

Parameters	No.	leptin (ng/ml)	p-value	adiponectin (ug/ml)	p-value	LAR	p-value
All	130	6.90	-	17.43	-	0.61	-
Sex							
Female	64	9.57	< 0.01	19.39	< 0.01	0.72	0.25
Male	66	4.31		15.52		0.49	
Age							
0-60 years	66	7.72	0.13	17.53	0.88	0.52	0.40
>60 years	64	6.06		17.32		0.69	
Body mass index							
<23.4 Kg/m^2	73	4.40	< 0.01	19.24	< 0.01	0.29	< 0.01
≥23.4 Kg/m^2	60	9.91		15.25		0.99	
Metabolic risk score							
0-1 (low risk)	61	5.55	0.07a	19.31	0.02a	0.38	0.07a
2-3 (moderate risk)	58	7.29	0.03b	15.84	0.85b	0.67	0.08b
4-6 (high risk)	11	12.32	<0.01c	15.40	0.18c	1.53	<0.01c
Groups							
No polyps	30	7.35	0.99d	19.49	0.02d	0.43	0.44d
Presence of CAP*	18	7.38		14.03		0.54	
Colorectal cancer	82	6.47		17.56		0.69	

LAR; Leptin to adiponectin ratio; a: comparing the 'low risk' group and the 'moderate risk' group, b: comparing the 'moderate risk' group and the 'high risk' group, c: comparing the 'low risk' and the 'high risk' group; d: comparing the 'no polyp' group and the 'presence of CAP' group; *CAP: Cancer Associated Polyps

Table 2. Leptin and Adiponectin Levels in 82 Colorectal Cancer Cases

Parameters	No.	leptin (ng/ml)	p-value	adiponectin (ug/ml)	p-value	LAR	p-value
All CRC	82	6.63	-	17.42	-	0.69	-
Site							
Colon	52	6.17	0.29	15.65	0.01	0.65	0.80
Rectum	30	7.43		20.49		0.73	
Side							
Left	71	7.06	0.06	17.01	0.27	0.75	0.23
Right	11	3.88		20.03		0.20	
T stage							
T1-2	17	7.92	0.25	13.41	0.03	1.34	0.03
T3-4	65	6.29		18.47		0.52	
N stage							
N0	44	6.46	0.92	17.65	0.91	0.72	0.72
N1-2	37	6.58		17.43		0.61	
Lymph node ratio							
0-0.35	73	6.54	0.83	17.1	0.42	0.62	0.22
>0.35	8	6.95		19.6		1.28	
M stage							
M0	67	6.63	0.99	16.58	0.05	0.67	0.63
M1	15	6.64		21.18		0.84	
AJCC stage							
Stage 1-2	43	6.76	0.82	16.64	0.38	0.78	0.53
Stage 3-4	39	6.49		18.29		0.58	
Differentiation							
Well	45	6.41		17.02		0.49	
Moderately	32	6.88	0.75[a]	17.25	0.69a	1.01	0.11a
Poorly	4	6.08		22.12		0.27	
Lymph-vascular invasion							
Present	56	6.85	0.50	17.32	0.95	0.7	0.88
Absent	25	5.98		17.46		0.65	

[a]comparing well differentiated group and moderately differentiated group

the most and that the pattern of all three parameters peaked in the polyp and the early T-stage CRC groups (Figure 2).

Discussion

We recently found a positive correlation between metabolic risk score and the likelihood of finding adenomatous polyps in patients who underwent a colonoscopy (Orannapalai et al., 2014). In the current study, two additional groups of adenocarcinoma patients were included in the analysis, and it was found that the distribution patterns of metabolic risk scores were the same between the non-invasive non-metastatic tumors (T1-2) and cancer-associated polyps, suggesting that the metabolic risk was associated with the initiation of CRC.

Leptin and adiponectin levels significantly changed with increasing BMI, indicating that the serum levels of these adipokines correlated well with the status of visceral adiposity. Consistent with this finding, there was a link between both markers and metabolic risk, as leptin trended upward and adiponectin trended downward with increasing metabolic risk score. We found that adipokine levels differed between the sexes, which was consistent with other reports and could be explained, at least in part, by the differences in body composition (Dalamaga et al., 2012). When the adenomatous polyps and control groups were compared, it was found that both adipokines were altered, but only adiponectin was significantly different between the two groups. Significantly lower adiponectin levels were also found when the early stage CRC group was compared with the controls. This finding is consistent with previous reports (Nakajima et al., 2010, Wei et al., 2011), which showed that adiponectin is a marker of adenoma development (Nakajima et al., 2010).

When adiponectin level was compared between early and invasive stages, it was found that the average level in the late T-stage group was higher and closer to the level in the control group. The average value of adiponectin was even higher in metastatic tumors although the difference was not statistically significant. As adiponectin reflects visceral adiposity, we expect this reversal of adiponectin level was likely due to alterations in nutritional status or a reduction in body fat composition in those patients with later stage tumors. Apart from CRC, decreased blood adiponectin has also been associated with an increased risk of other cancers, including renal cell carcinoma, breast cancer, endometrial carcinoma, prostate cancer, and liver cancer (Dalamaga et al., 2012).

There is one notable limitation of our study. There was a relatively small number of cases in the adenomatous polyp group. However, as the adipokine level in the early T-stage group was close to that of the adenomatous polyp, our conclusions relied not only on the significant differences but also the consistent pattern among the groups of similar tumor stage. Future studies should be directed at designating a high adiponectin level as an indication for CRC screening in individuals without other clinical indications.

In conclusion, our study evaluated serum levels of two adipokines, leptin and adiponectin, in premalignant and malignant lesions of the large intestine. We found that adiponectin was significantly lower in patients with colorectal adenoma and early stage adenocarcinoma, with adiponectin levels returning to near-normal levels in invasive and metastatic cancers. In addition, as adiponectin levels decreased, the metabolic risk score increased. We suggest that adiponectin can be used as a marker to link metabolic risk and colorectal cancer development.

Acknowledgements

The work was supported by the Thailand Research Fund (MRG5208118). Dave Patterson edited English Language in the manuscript.

References

Alberti KG, Zimmet P, Shaw J (2006). Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med*, **23**, 469-80.

Aleksandrova K, Jenab M, Bueno-de-Mesquita HB, et al (2014). Biomarker patterns of inflammatory and metabolic pathways are associated with risk of colorectal cancer: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Epidemiol*, **29**, 261-75.

Arnold CN, Goel A, Blum HE, Boland CR (2005). Molecular pathogenesis of colorectal cancer: implications for molecular diagnosis. *Cancer*, **104**, 2035-47.

Attele AS, Shi ZQ, Yuan CS (2002). Leptin, gut, and food intake. *Biochem Pharmacol*, **63**, 1579-83.

Bonakdaran S, Kharaqani B (2014). Association of serum uric acid and metabolic syndrome in type 2 diabetes. *Curr Diabetes Rev*, **10**, 113-7.

Cowley MA, Smart JL, Rubinstein M, et al (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, **411**, 480-4.

Dalamaga M, Diakopoulos KN, Mantzoros CS (2012). The role of adiponectin in cancer: a review of current evidence. *Endocr Rev*, **33**, 547-94.

Doyle SL, Donohoe CL, Lysaght J, Reynolds JV (2012). Visceral obesity, metabolic syndrome, insulin resistance and cancer. *Proc Nutr Soc*, **71**, 181-9.

Erarslan E, Turkay C, Koktener A, et al (2009). Association of visceral fat accumulation and adiponectin levels with colorectal neoplasia. *Dig Dis Sci*, **54**, 862-8.

Gialamas SP, Sergentanis TN, Antonopoulos CN, et al (2013). Circulating leptin levels and risk of colorectal cancer and adenoma: a case-control study and meta-analysis. *Cancer Causes Control*, **24**, 2129-41.

Gonullu G, Kahraman H, Bedir A, Bektas A, Yucel I (2010). Association between adiponectin, resistin, insulin resistance, and colorectal tumors. *Int J Colorectal Dis*, **25**, 205-12.

Green ED, Maffei M, Braden VV, et al (1995). The human obese (OB) gene: RNA expression pattern and mapping on the physical, cytogenetic, and genetic maps of chromosome 7. *Genome Res*, **5**, 5-12.

Greene FL (2002). The american joint committee on cancer: updating the strategies in cancer staging. *Bull Am Coll Surg*, **87**, 13-5.

Hardwick JC, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP (2001). Leptin is a growth factor for colonic epithelial cells. *Gastroenterol*, **121**, 79-90.

Kadowaki T, Yamauchi T (2005). Adiponectin and adiponectin receptors. *Endocr Rev*, **26**, 439-51.

Kritsanasakul A, Boonpipattanapong T, Wanitsuwan W, et al (2012). Impact of lymph node retrieval on surgical outcomes in colorectal cancers. *J Surg Oncol*, **106**, 238-42.

Kumor A, Daniel P, Pietruczuk M and Malecka-Panas E (2009). Serum leptin, adiponectin, and resistin concentration in colorectal adenoma and carcinoma (CC) patients. *Int J Colorectal Dis*, **24**, 275-81.

Lubkowska A, Radecka A, Bryczkowska I, et al (2015). Serum Adiponectin and Leptin Concentrations in Relation to Body Fat Distribution, Hematological Indices and Lipid Profile in Humans. *Int J Environ Res Public Health*, **12**, 11528-48.

Lukanova A, Soderberg S, Kaaks R, Jellum E, Stattin P (2006). Serum adiponectin is not associated with risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev*, **15**, 401-2.

Nakajima TE, Yamada Y, Hamano T, et al (2010). Adipocytokines as new promising markers of colorectal tumors: adiponectin for colorectal adenoma, and resistin and visfatin for colorectal cancer. *Cancer Sci*, **101**, 1286-91.

Orannapalai N, Attawettayanon W, Kanngern S, Boonpipattanapong T, Sangkhathat S (2014). Predicting the occurrence of cancer-associated colorectal polyp using a metabolic risk score. *Mol Clin Oncol*, **2**, 124-8.

Prolo P, Wong ML, Licinio J (1998). Leptin. *Int J Biochem Cell Biol*, **30**, 1285-90.

Riondino S, Roselli M, Palmirotta R, et al (2014). Obesity and colorectal cancer: role of adipokines in tumor initiation and progression. *World J Gastroenterol*, **20**, 5177-90.

Seoane-Collazo P, Ferno J, Gonzalez F, et al (2015). Hypothalamic-autonomic control of energy homeostasis. *Endocrine*, **50**, 276-91.

Singh SP, Kar SK, Panigrahi MK, et al (2013). Profile of patients with incidentally detected nonalcoholic fatty liver disease (IDNAFLD) in coastal eastern India. *Trop Gastroenterol*, **34**, 144-52.

Trujillo ME, Scherer PE (2005). Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med*, **257**, 167-75.

Vazzana N, Riondino S, Toto V, et al (2012). Obesity-driven inflammation and colorectal cancer. *Curr Med Chem*, **19**, 5837-53.

Wang D, Chen J, Chen H, et al (2012). Leptin regulates proliferation and apoptosis of colorectal carcinoma through PI3K/Akt/mTOR signalling pathway. *J Biosci*, **37**, 91-101.

Wei C, Ford A, Hunt L, Crowne EC, Shield JP (2011). Abnormal liver function in children with metabolic syndrome from a UK-based obesity clinic. *Arch Dis Child*, **96**, 1003-7.

Yamauchi T, Kadowaki T (2008). Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int J Obes*, **32**, 13-8.

Zekri AR, Bakr YM, Ezzat MM, Zakaria MS, Elbaz TM (2015). Circulating levels of adipocytokines as potential biomarkers for early detection of colorectal carcinoma in Egyptian patients. *Asian Pac J Cancer Prev*, **16**, 6923-8.

Zhang L, Wen K, Han X, Liu R, Qu Q (2015). Adiponectin mediates antiproliferative and apoptotic responses in endometrial carcinoma by the AdipoRs/AMPK pathway. *Gynecol Oncol*, **137**, 311-20.

Zhang Y, Proenca R, Maffei M, et al (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, **372**, 425-32.